

# **iBiology.org Teaching Tools**

## **Chaitan Khosla's Lecture Part 3:**

### **Vectorial Specificity of Assembly Lines**

Teaching Tools were prepared by Becky Anderson and Ben Barsi-Rhyne.

#### **Contents**

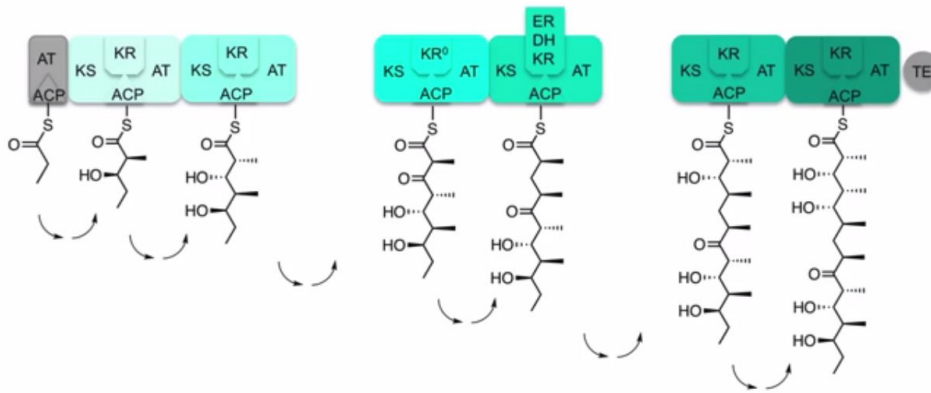
1. Keywords and Terms
  2. Lecture Notes
  3. Review Questions
  4. Answers to Review Questions
  5. Discussion Questions
  6. Answers to Discussion Questions
  7. Questions for Discussion Paper
  8. Answers to Questions for Discussion Paper
- 

#### **1. Keywords and Terms**

vectorial specificity, docking sites, reverse translocation, reactive intermediates, stuttering module

#### **2. Lecture Notes**

## How to Channel Reactive Intermediates Vectorially on a Polyketide Assembly Line?

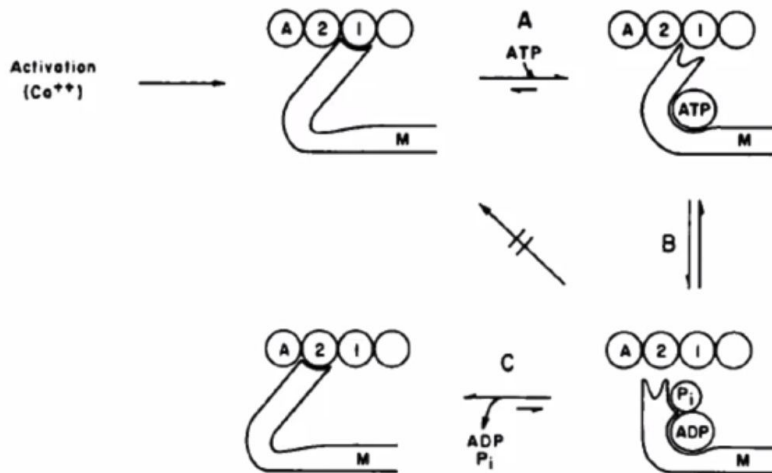


ACP = acyl carrier protein  
 AT = acyltransferase  
 KS = ketosynthase  
 KR = ketoreductase  
 DH = dehydratase  
 ER = enoylreductase



**2:37 min**

There are a large number of orphan polyketide assembly lines that have unknown mechanisms. Understanding the rules for polyketide synthesis in a well-studied process like the one shown on the left will lay the foundation for understanding orphan polyketide assembly lines.

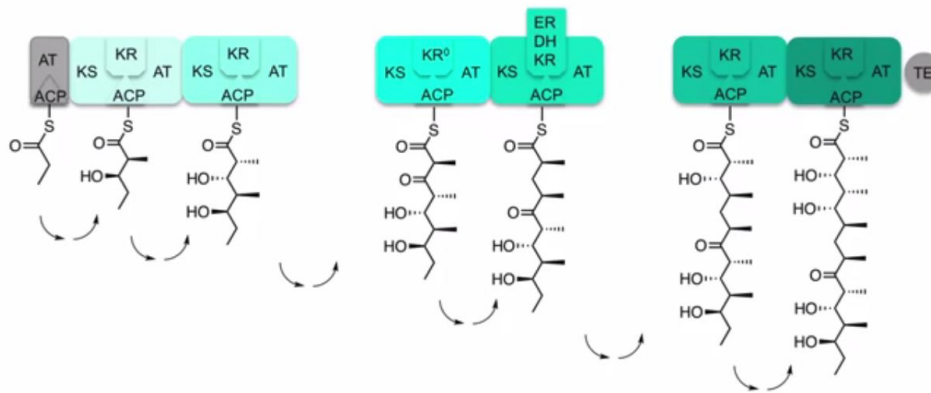


iBiology.org

**7:28 min**

“Coupled vectorial systems differ from ordinary enzymes in that they undergo large changes in substrate specificity in different states.” –William Jencks  
 The movement of myosin on actin fibers is an example of a coupled vectorial system. Movement is achieved by altering the structure of myosin at different points in the cycle to increase or decrease its specificity for actin.

## How to Channel Reactive Intermediates Vectorially on a Polyketide Assembly Line?



ACP = acyl carrier protein  
 AT = acyltransferase  
 KS = ketosynthase  
 KR = ketoreductase  
 DH = dehydratase  
 ER = enoylreductase

• Specificity change occurs at level of protein-protein interactions

• Specificity of KS-AT core for ACP establishes directionality

Khosla, et al. (2014) *Biochemistry*



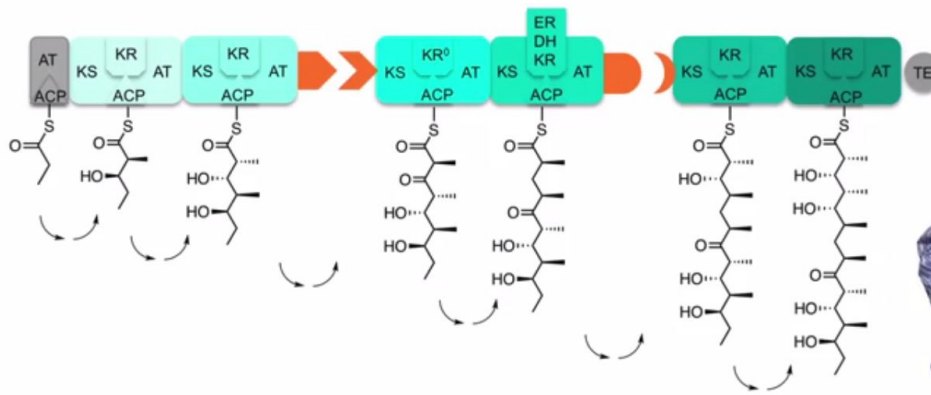
iBiology.org

10:39 min

Two hypothesis for vectorial polyketide assembly are:

- Specificity changes occur between proteins
- Directionality is the result of KS-AT specificity for ACP

## Importance of Protein-Protein Interactions



ACP = acyl carrier protein  
AT = acyltransferase  
KS = ketosynthase  
KR = ketoreductase  
DH = dehydratase  
ER = enoylreductase

Gokhale, et al. (1999) *Science*

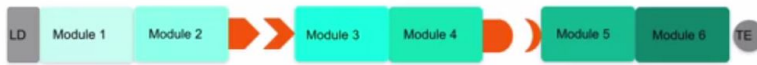


iBiology.org

**11:57 min**

Docking sites that do not play a role in the core enzymology are responsible for determining directionality.

## Importance of Protein-Protein Interactions



$$k_{\text{cat}} = 0.3 \text{ min}^{-1}$$
$$K_{\text{D}} = 1.1 \text{ } \mu\text{M}$$



$$k_{\text{cat}} < 0.03 \text{ min}^{-1}$$

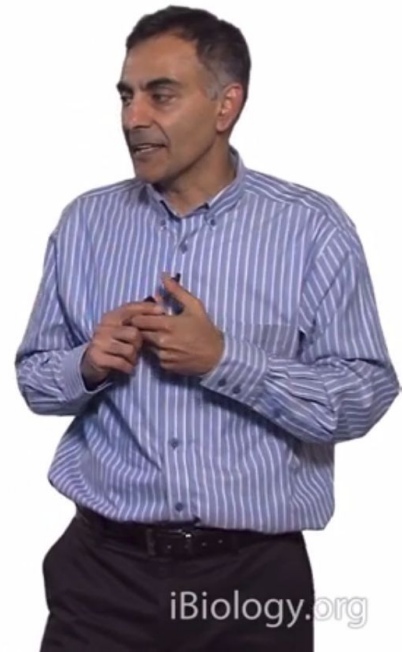


$$k_{\text{cat}} < 0.03 \text{ min}^{-1}$$



$$k_{\text{cat}} = 0.7 \text{ min}^{-1}$$
$$K_{\text{D}} = 2.1 \text{ } \mu\text{M}$$

Tsuji, et al. (2001) *Biochemistry*



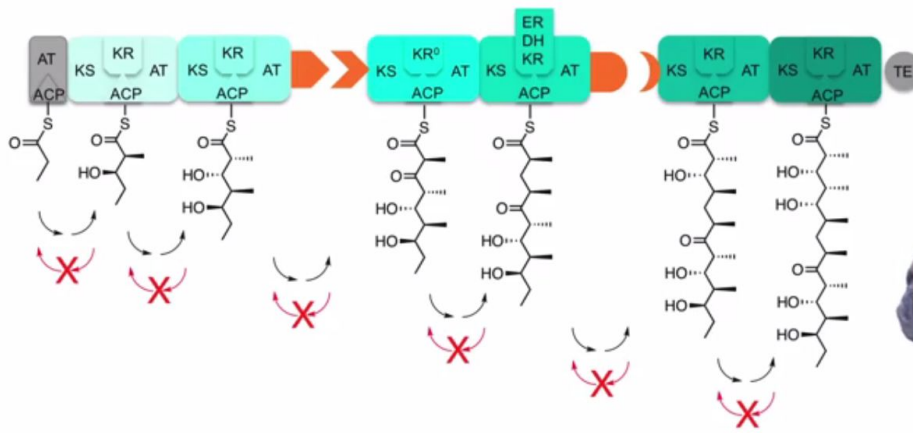
### 13:03 min

Mixing and matching docking sites between modules provides evidence for the protein-protein interaction specificity hypothesis.

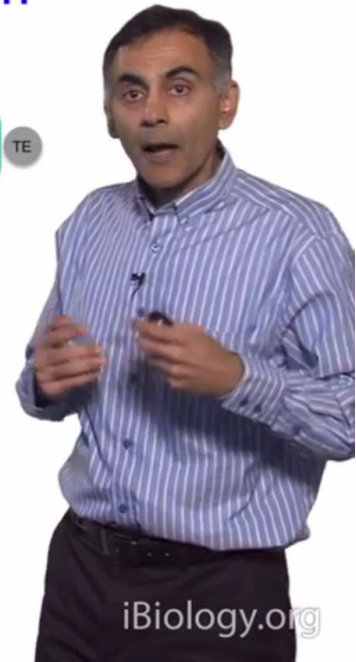
When docking sites are paired correctly turnover is relatively normal even if the docking sites are from another module.

When docking sites are incorrectly paired, turnover is reduced.

## What Precludes Reverse Chain Translocation?



ACP = acyl carrier protein  
 AT = acyltransferase  
 KS = ketosynthase  
 KR = ketoreductase  
 DH = dehydratase  
 ER = enoylreductase

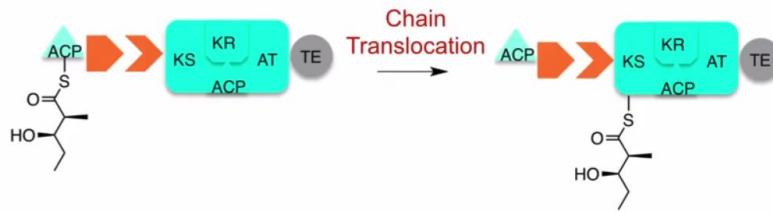


iBiology.org

**15:01 min**

Directionality is established by the protein-protein interactions mentioned on the previous slide, but these interactions do nothing to prevent reverse chain translocation.

## KS-ACP Recognition During Chain Translocation



$k_{cat}/K_M$  ( $\text{min}^{-1}\mu\text{M}^{-1}$ )

ACP2	390
ACP4	2

ACP = acyl carrier protein  
 AT = acyltransferase  
 KS = ketosynthase  
 KR = ketoreductase  
 DH = dehydratase  
 ER = enoylreductase

Wu, et al. (2002) *Biochemistry*



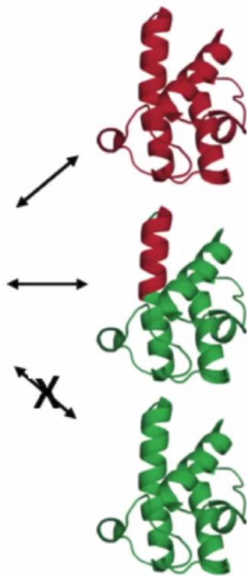
### 16:28 min

Interactions outside of the docking sites between KS and ACP prevent backward movement through the assembly line.

This is demonstrated by replacing ACP2 with ACP4 and observing the decrease in specificity.

## KS-ACP Recognition During Chain Translocation

	$k_{cat}/K_M$ ( $\text{min}^{-1}\mu\text{M}^{-1}$ )
ACP2	390
ACP4	2



ACP = acyl carrier protein  
 AT = acyltransferase  
 KS = ketosynthase  
 KR = ketoreductase  
 DH = dehydratase  
 ER = enoylreductase

Kapur, et al. (2010) *PNAS*



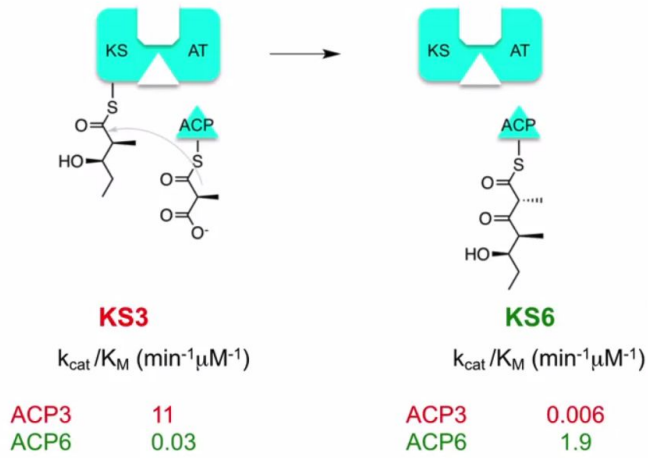
iBiology.org

**17:42 min**

Different portions of protein dictate specificity for different events.

The red protein does a good job of talking to the acceptor protein while the green protein does not. The red and green chimeric protein is just as good at talking to the acceptor protein as the red protein.

## KS-ACP Recognition During Chain Elongation



ACP = acyl carrier protein  
 AT = acyltransferase  
 KS = ketosynthase  
 KR = ketoreductase  
 DH = dehydratase  
 ER = enoylreductase

Chen, et al. (2006) *JACS*

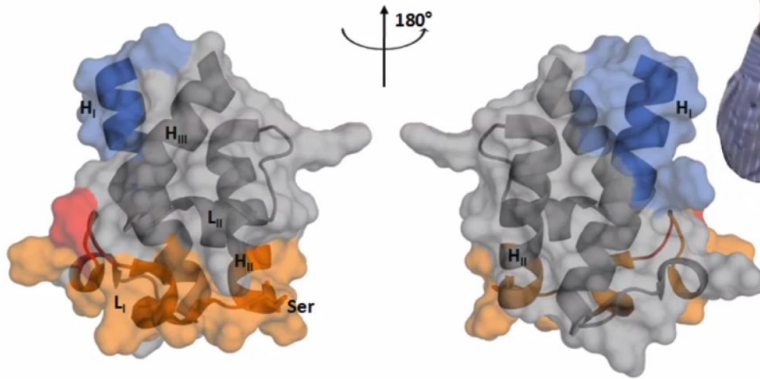


### 18:47 min

Replacing ACP in chain elongation can also have large effects on the specificity constant.

Chimeric versions of these proteins have been made and show what portions of the protein are responsible for recognition in the same manner showed on the previous slide.

## Chain **Elongation** & **Translocation** are Controlled by Different Faces of the Acyl Carrier Protein



Kapur, et al. (2010) *PNAS*



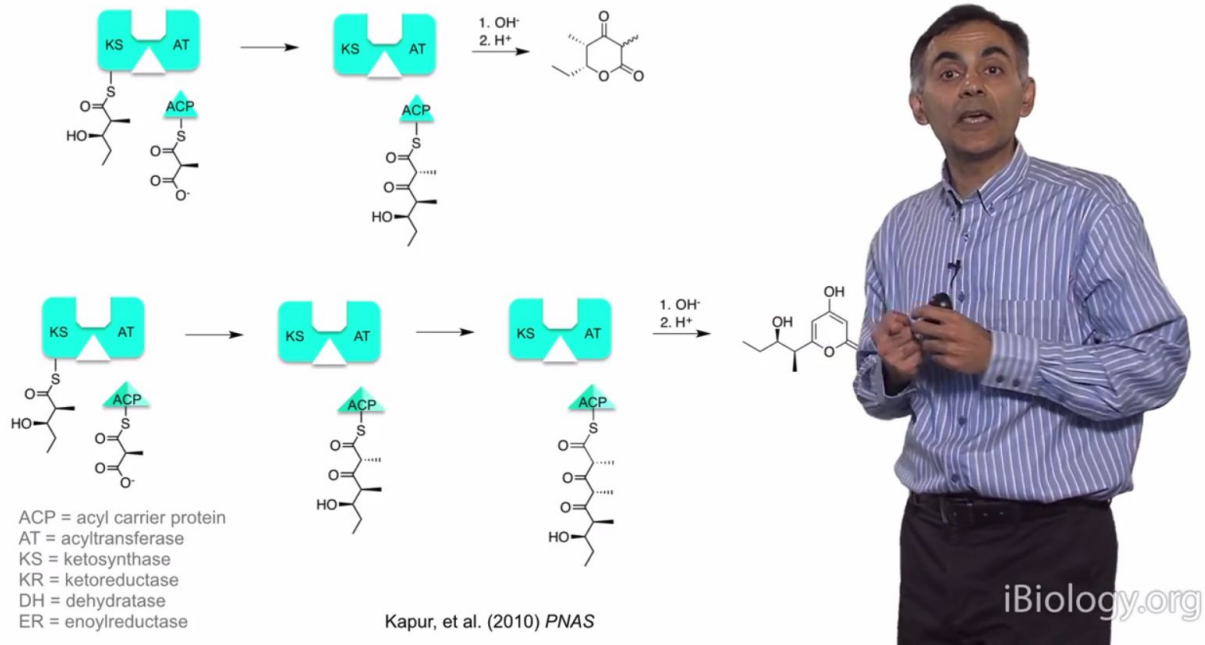
**19:44 min**

Elongation and translocation are sequential events that require specificity determined by different faces of the protein.

The blue face controls translocation.

The orange face controls elongation.

## Rationally Designed Stuttering Module



iBiology.org

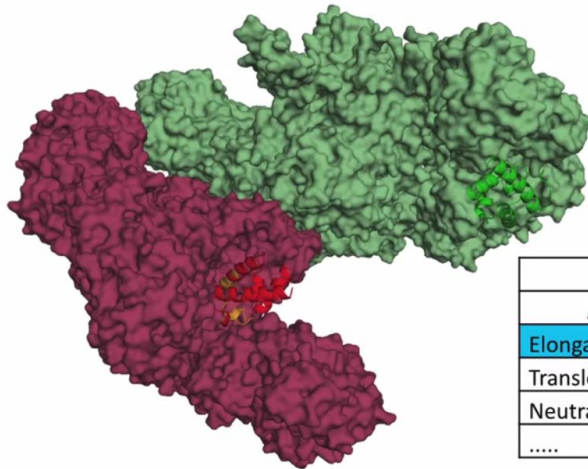
**20:39 min**

A specific carrier protein can only recognize the next module in the assembly line.

The first and second ACPs at the top of this figure are not interchangeable and thus the growing polyketide chain cannot go backwards.

A stutter is generated by engineering an ACP that can recognize both modules. This allows two rounds of elongation to occur before stopping.

## Visualizing Changes in Specificity along the Catalytic Cycle



ACP state	
ACP2	ACP3
Elongation	Neutral
Translocation	Neutral
Neutral	Elongation
.....	.....



iBiology.org

**23:34 min**

The model to the left represents modules two and three of the assembly line.

Initially the red carrier protein (ACP2) is in an elongation state and the green carrier protein is in a neutral state.

Then the red carrier protein switches to the translocation state. This hands off the polyketide to the green carrier protein (ACP3) that then undergoes a second round of elongation.

### 4. Review Questions

1. What two hypotheses are used to explain vectorial polyketide assembly?
2. How do docking sites influence polyketide assembly?
3. How is reverse translocation prevented?
4. What protein is responsible for recognizing the next module in the assembly line?
  - a. ACP
  - b. AT

- c. KS
  - d. KR
  - e. DH
  - f. ER
5. Briefly describe the catalytic cycle described in the last section of this lecture.

## 5. Answers to Review Questions

1.
  - a. Specificity occurs at the level of protein-protein interactions.
  - b. Specificity of KS-AT core for ACP determines directionality.
2. Docking sites between modules allows for efficient movement of the growing polyketide chain from one module to another. Mismatched modules prevent the efficient movement.
3. KS and ACP interactions outside of the docking site prevent backwards movement.
4. A
5. Initially the red carrier protein (ACP2) is in an elongation state and the green carrier protein is in a neutral state. Then the red carrier protein switches to the translocation state. This hands off the polyketide to the green carrier protein (ACP3) that then undergoes a second round of elongation.

## 5. Discussion Questions

1. Can you think of an interesting experiment involving polyketide assembly pathways that was not presented in the lectures?
2. Did this lecture leave you with any unanswered questions?
3. What do you think is a major hurdle to engineering pathways like this to create a non-natural product?

## 6. Answers to Discussion Questions

1. An interesting and probably very difficult experiment might be to take the stand-alone acyltransferase mentioned in the previous lecture and try to adapt it to fit into the assembly line like the native protein. If this can be done with an enzyme that has a high turnover it might be interesting to do it with an acyltransferase that has a low turnover. The hope would be that fitting the exogenous acyltransferase into the pathway and getting a reasonable rate of product formation would allow many different acyltransferases to be used not just the ones with really high rates.
2. Do the other modules of the assembly line behave in the same manner as modules two and three depicted? Do earlier and later modules have any differences outside of the docking sites?
3. One major hurdle is identifying proteins that are similar enough that they can be used in the assembly line, but different enough to contribute some interesting chemistry. The example given in the lecture was using an acyltransferase that favored malonyl-CoA over methylmalonyl-CoA. If enzymes existed in nature that favored other interesting functional groups, novel chemistry could be achieved.

## 7. Questions for Discussion Paper

Walker, MC. et al. Expanding the fluorine chemistry of living systems using engineered polyketide synthase pathways.

Science. 2013 Sep 6; 341(6150): 1089–1094.

1. What was the overall goal of this research? Why would achieving this goal be useful?
2. How are the experiments shown in figures 2A and 2B similar? What is different about them?
3. Describe the experimental set-up depicted in Figure 3. What are the two main results from this experiment?
4. What is the purpose of making the different mutations shown in Figures 4C and D? How do these mutations affect the DEBS modules, and how is this useful?

5. What traits of *E. coli* are important for the *in vivo* chain extension reactions using fluoromalonyl-CoA?

## 8. Answers to Questions for Discussion Paper

1. The goal of this research was to develop a method of introducing fluorine into small molecules that could serve as building blocks for complex fluorinated products. Specifically, fluoromalonyl-CoA could act as an extender unit for polyketide biosynthesis, enabling the introduction of fluorine into structurally complex and biologically active compounds. This would be useful because many pharmaceutical compounds contain at least one fluorine atom. Organofluorines are also used in diverse fields including diagnostics and agriculture.
2. They are similar because they both result in the production of fluoromalonyl-CoA. However, they use different pathways to create this product. In 2A, a two-step reaction is used starting from fluoroacetate. This is important because fluoroacetate is produced naturally by certain bacteria. In 2B, CoA is directly ligated to fluoromalonate.
3. This experiment tests whether a fluorinated extender can be used in a chain elongation reaction. Fluoromalonyl-CoA was used as an extender to lengthen an acetyl-CoA starter. This reaction was catalyzed by a simple ketosynthase NphT7 and the ketoreductase PhaB. The two main results are that the ketosynthase could efficiently use fluoromalonyl-CoA as an extender to create acetofluoroacetyl-CoA, and that the ketoreductase was capable of reducing this fluorinated product. Since chain elongation and ketoreduction are key steps in the catalytic cycle of polyketide synthases, it was important to verify that both of these processes could occur with the fluorinated substrate.
4. These mutations were made in order to alter the specificity of the DEBS modules. Normally these modules have a high preference for methylmalonyl-CoA over fluoromalonyl-CoA. However, the mutations shown in these figures confer a preference for fluoromalonyl-CoA. This is useful because by combining these mutated and normal DEBS modules, fluorine could be incorporated in a site-specific manner. Importantly, in 4D they find that a two-step chain elongation reaction can occur even when fluorine is incorporated in the first step, indicating that fluorinated intermediates can be tolerated in downstream reactions.
5. The DEBS modules have a preference for methylmalonyl-CoA over fluoromalonyl-CoA, however *E. coli* contain almost no methylmalonyl-CoA. While

they do have a sizable pool of malonyl-CoA, this is much less efficiently incorporated by the DEBS modules than fluoromalonyl-CoA is. Therefore, fluoromalonyl-CoA would face little competition as an extender unit. Another useful aspect of performing *in vivo* chain extension reactions is that *E. coli* continually generate ATP through normal metabolic processes, whereas ATP needed to be added to all of the *in vitro* reactions.